IT IS CLAIMED:

1. A purified β-secretase protein.

2. The purified protein of claim 1, characterized by a specific activity of at least about 0.1 x 10^5 nM/h/μg protein in a C125-SW substrate assay.

3. The β-secretase protein of claim 1, wherein said specific activity is at least 0.5 x 10^6 nM/h/μg protein.

4. The protein of claim 1, wherein said protein includes the amino acid sequence SEQ ID NO: 43 [46-501].

5. The protein of claim 1, wherein said protein has the amino acid sequence SEQ ID NO: 58 [46-452].

6. The protein of claim 1, wherein said protein has the amino acid sequence SEQ ID NO: 2 [22-501].

7. The protein of claim 1, wherein said protein is derived from human cells or human tissue.

8. The protein of claim 1, wherein said protein has an amino acid sequence selected from the group consisting of SEQ ID NO: 67 [58-501] and SEQ ID NO: 69 [63-501].

9. The protein of claim 1, wherein said protein is isolated from a mouse.

10. The protein of claim 9, wherein said protein has the sequence SEQ ID NO: 65.

11. The protein of any of claims 1-10, wherein said protein is produced by a heterologous cell.

12. A crystalline protein composition formed from a purified β-secretase protein.

13. The crystalline protein composition of claim 12, wherein said purified protein is characterized by an ability to bind to the β-secretase inhibitor substrate P10-P4'sta D→V which is at least equal to an ability exhibited by a protein having the amino acid sequence SEQ ID NO: 70 [46-419], when said proteins are tested for binding to said substrate under the same conditions.

14. The crystalline protein composition of claim 12, wherein said composition is formed from any of the proteins of claims 1-10.

15. The crystalline protein composition of claim 14, wherein said composition further includes a β-secretase substrate or inhibitor molecule.

16. The crystalline protein composition of claim 15, wherein said β-secretase substrate or inhibitor molecule is added to said crystalline protein by soaking.

17. The crystalline protein composition of claim 15, wherein said β-secretase inhibitor is a peptide having fewer than about 15 amino acids and comprises the sequence SEQ ID NO: 73 (EVM[sta]VAEF; P4-P4'sta D->V), including conservative substitutions thereof.

18. The crystalline protein composition of claim 15, wherein said β-secretase inhibitor has the sequence SEQ ID NO: 72 [P10-P4'sta D->V], including conservative substitutions thereof.

19. The crystalline protein composition of claim 15, wherein said β-secretase inhibitor includes fewer than about 15 amino acids and comprises the sequence EVM[hydroxyethylene]AEF, including conservative amino acid substitutions thereof.

20. The crystalline protein composition of claim 15, wherein said β-secretase inhibitor is characterized by a K_i of no more than about 1 mM.

21. The crystalline protein composition of claim 15, wherein said β-secretase inhibitor is characterized by a K_i of no more than about 50 μM.

22. An isolated protein, comprising a polypeptide that (i) is fewer than about 480 amino acid residues in length, (ii) includes an amino acid sequence that is at least 90% identical to SEQ ID NO: 58 [46-452] including conservative substitutions thereof, and (iii) exhibits β-secretase activity, as evidenced by an ability to cleave MBP-C125sw.

23. The protein of claim 22, wherein said amino acid sequence is at least 95% identical to SEQ ID NO: 58 [46-452].

24. The protein of claim 23, wherein said polypeptide includes the amino acid sequence SEQ ID NO: 58 [46-452].

25. The protein of claim 24, wherein said protein consists of a polypeptide having the sequence SEQ ID NO: 58 [46-452].

26. The protein of claim 22, wherein said polypeptide includes the amino acid sequence SEQ ID NO: 43 [46-501].

27. The protein of claim 22, wherein said polypeptide includes the amino acid sequence SEQ ID NO: 74 [22-452].

28. The protein of claim 22, wherein said polypeptide includes the amino acid sequence of SEQ ID NO: 66 [22-501].

29. The protein of claim 22, wherein said polypeptide is derived from human cells or human tissue.

30. The protein of claim 22, wherein said polypeptide is derived from mouse cells or mouse tissue.

31. The protein of claim 30, wherein said polypeptide has the sequence SEQ ID NO: 65.

32. An isolated protein, comprising a polypeptide that (i) is fewer than about 480 amino acid residues in length, (ii) includes an amino acid sequence that is at least 90% identical to SEQ ID NO: 75 [63-423] including conservative substitutions thereof, and (iii) exhibits β-secretase activity, as evidenced by an ability to cleave MBP-C125sw.

33. The protein of claim 32, wherein said polypeptide includes the amino acid sequence of SEQ ID NO: 75 [63-423].

34. The protein of claim 33, wherein said polypeptide has the SEQ ID NO: 75.

35. The protein of claim 32, wherein said polypeptide is derived from human cells or human tissue.

36. The protein of any of claims 22-35, wherein said protein is expressed by a heterologous cell.

37. A composition comprising any of the proteins of claims 1-10 or 22-35, and a β-secretase substrate or inhibitor molecule.

38. The composition of claim 37, wherein said β-secretase substrate is selected from the group consisting of MBP-C125wt and MBP-C125sw.

39. The composition of claim 37, wherein said β-secretase substrate is selected from the group consisting of APP, APPsw, and β-secretase-cleavable fragments thereof.

40. The composition of claim 39, wherein said β-secretase-cleavable fragment is selected from the group consisting of SEVKMDAEF (P5-P4'wt), SEVNLDAEF (sw), SEVKLDAEF, SEVKFDAEF, SEVNFDAEF, SEVKMAAEF, SEVNLAAEF, SEVKLAAEF; SEVKMLAEF, SEVNLLAEF, SEVKLLAEF, SEVKFAAEF, SEVNFAAEF, SEVKFLAEF, and SEVNFLAEF.

41. The composition of claim 37, wherein said β-secretase inhibitor is a peptide having fewer than about 15 amino acids and comprises the sequence EVM[sta]VAEF (P4-P4'sta D->V), including conservative substitutions thereof.

42. The composition of claim 41, wherein said β-secretase inhibitor has the sequence SEQ ID NO: 72 (P10-P4'sta D->V), including conservative substitutions thereof.

43. The composition of claim 37, wherein said β-secretase inhibitor includes fewer than about 15 amino acids and comprises the sequence EVM[hydroxyethylene]AEF, including conservative amino acid substitutions thereof.

44. The composition of claim 37, wherein said β-secretase inhibitor has a K_i of no more than about 1 μM.

45. The composition of claim 37, wherein said β-secretase inhibitor is labeled with a detectable reporter molecule.

46. The composition of claim 45, wherein said β-secretase inhibitor and said β-secretase protein are present in a ligand binding assay.

47. An antibody which binds specifically to any of the protein compositions of claims 1-10 or 25-35, wherein said antibody further lacks significant immunoreactivity with a protein having the sequence SEQ ID NO: 2 [1-501].

48. An isolated nucleic acid, comprising a sequence of nucleotides that encodes the β-secretase protein of any of claims 1-10 or 22-35, or a complementary sequence of any of such nucleotides.

49. The isolated nucleic acid of claim 48, wherein said sequence of nucleotides encodes a protease having an amino acid sequence at least 95% identical to the sequence SEQ ID NO: 75 [63-423], including conservative substitutions thereof.

50. The isolated nucleic acid of claim 48, wherein said sequence of nucleotides encodes a protease having an amino acid sequence at least 95% identical to the sequence SEQ ID NO: 58 [46-501], including conservative substitutions thereof.

51. A expression vector, comprising

the isolated nucleic acid of claim 48, 49 or 50; and

operably linked to said nucleic acid, regulatory sequences effective for expression of the nucleic acid in a selected host cell.

52. The recombinant expression vector of claim 51, wherein said vector is suitable for transfection of a bacterial cell.

53. A heterologous cell transfected with the vector of claim 51, wherein said cell expresses a biologically active β-secretase.

54. The cell of claim 53, wherein said cell is a eukaryotic cell.

55. The cell of claim 53, wherein said cell is a bacterial cell.

56. The cell of claim 53, wherein said cell is an insect cell.

57. The cell of claim 53, wherein said cell is a yeast cell.

58. A method of producing a recombinant β-secretase enzyme, comprising culturing a cell according to any of claims 53-57 under conditions to promote growth of said cell, and subjecting an extract or cultured medium from said cell to an affinity matrix.

59. The method of claim 58, wherein said affinity matrix contains a β-secretase inhibitor molecule.

60. The method of claim 59, wherein said inhibitor molecule is P10-P4'staD->V.

61. The method of claim 58, wherein said matrix contains an antibody characterized by an ability to bind β-secretase.

62. The method of claim 58, wherein said antibody binds specifically to any of the protein compositions of claims 1-10 or 22-35.

63. The method of claim 58, wherein said antibody further lacks significant immunoreactivity with a protein having the sequence SEQ ID NO: 2 [1-501].

64. A heterologous cell, comprising

(i) a nucleic acid molecule encoding a biologically active β-secretase protein according to any of claims 1-10 or 22-35, or the complementary sequence of said nucleic acid molecule;

(ii) a nucleic acid molecule encoding a β-secretase substrate molecule; and

(iii) operatively linked to (i) and (ii), a regulatory sequence effective for expression of said nucleic acid molecules in said cell.

65. The cell of claim 64, wherein said nucleic acid encoding said β-secretase protein is heterologous to said cell.

66. The cell of claim 64, wherein both said nucleic acids encoding said β-secretase protein encoding said β-secretase substrate molecule are heterologous to said cell.

67. The cell of claim 64, wherein said β-secretase substrate molecule is selected from the group consisting of APPwt, APPsw, and ß-secretase cleavable fragments thereof.

68. The cell of claim 64, wherein said β-secretase substrate is selected from the group consisting of MBP-C125wt and MBP-C125sw.

69. The cell of claim 64, wherein said β-secretase-cleavable fragment is selected from the group consisting of SEVKMDAEF (P5-P4'wt), SEVNLDAEF (sw), SEVKLDAEF, SEVKFDAEF, SEVNFDAEF, SEVKMAAEF, SEVNLAAEF, SEVKLAAEF;

SEVKMLAEF, SEVNLLAEF, SEVKLLAEF, SEVKFAAEF, SEVNFAAEF, SEVKFLAEF, and SEVNFLAEF.

70. A method of screening for compounds that inhibit Aβ production, comprising contacting a β-secretase polypeptide with (i) a test compound and (ii) a β-secretase substrate, and selecting the test compound as capable of inhibiting Aβ production if said β-secretase polypeptide exhibits less β-secretase activity in the presence of said compound than in the absence of said compound.

71. The method of claim 70, wherein said active β-secretase polypeptide is according to any of claims 1-10 or 22-35.

72. The method of claim 70, wherein said active β-secretase polypeptide has a sequence selected from the group consisting of SEQ ID NO: 43 [46-501] and SEQ ID NO: 58 [46-452].

73. The method of claim 70, wherein said active β-secretase polypeptide has the sequence SEQ NO: 2 [1-501].

74. The method of claim 70, wherein said β-secretase polypeptide and said substrate are produced by a cell according to any of claims 64-69.

75. The method of claim 70, which further includes administering said test compound to a mammalian subject having Alzheimer's disease or Alzheimer's disease-like pathology, and selecting said compound as a therapeutic agent candidate if, following such administration, said subject maintains or improves cognitive ability or said subject shows reduce plaque burden.

76. The method of claim 75, wherein said subject is a transgenic mouse.

77. The method of claim 76, wherein said mouse is a PDAPP mouse or a Swedish mouse.

78. The method of claim 70, wherein said β-secretase substrate is selected from the group consisting of MBP-C125wt, MBP-C125sw, APP, APPsw, and ß-secretase-cleavable fragments thereof.

79. The method of claim 78, wherein said β-secretase-cleavable fragment is selected from the group consisting of SEVKMDAEF (P5-P4'wt), SEVNLDAEF (sw), SEVKLDAEF, SEVKFDAEF, SEVNFDAEF, SEVKMAAEF, SEVNLAAEF, SEVKLAAEF; SEVKMLAEF, SEVNLLAEF, SEVKLLAEF, SEVKFAAEF, SEVNFAAEF, SEVKFLAEF, and SEVNFLAEF.

80. A method of screening for compounds that inhibit Aβ production, comprising measuring binding of a β-secretase polypeptide of any of claims 1-10 or 22-35 with a β-secretase inhibitor compound in the presence of a test compound, and selecting the test compound as β-secretase active-site binding compound, if binding of the inhibitor in the presence of said test compound is less than binding of the inhibitor in the absence of said test compound.

81. The method of claim 80, wherein said inhibitor compound is radiolabeled.

82. The method of claim 80, wherein said β-secretase inhibitor is a peptide having fewer than about 15 amino acids and comprises the sequence EVM[sta]VAEF (P4-P4'sta D->V), including conservative substitutions thereof.

83. The method of claim 80, wherein said β-secretase inhibitor has the sequence (P10-P4'sta D->V), including conservative substitutions thereof.

84. The method of claim 80, wherein said β-secretase inhibitor includes fewer than about 15 amino acids and comprises the sequence EVM[hydroxyethylene]AEF, including conservative amino acid substitutions thereof.

85. The method of claim 80, wherein said β-secretase inhibitor has a Ki with respect to ß-secretase of less than about 50 μM.

86. A β-secretase inhibitor compound selected according to the method of any of claims 70-85.

87. The inhibitor of claim 86, wherein said compound is selected from a phage display selection system.

88. The compound of claim 87, wherein the phage display selection system is biased for the sequence [P10-P4'D→V].

89. A β-secretase inhibitor, comprising a peptide having the sequence EVM[sta]VAEF (P4-P4'sta D->V), including conservative substitutions thereof.

90. The β-secretase inhibitor of claim 89, having the sequence SEQ ID NO: 72 (P10-P4'sta D->V).

91. A screening kit, comprising

a β-secretase protein according to any of claims 1-10 or 22-35,

a cleavable β-secretase substrate, and

means for detecting cleavage of said substrate by β-secretase.

92. The screening kit of claim 91, wherein said β-secretase protein is present in a heterologous cell.

93. The screening kit of claim 91, wherein said β-secretase substrate molecule is selected from the group consisting of APPwt, APPsw, and β-secretase cleavable fragments thereof.

94. The screening kit of claim 93, wherein said β-secretase-cleavable fragment is selected from the group consisting of SEVKMDAEF (P5-P4'wt), SEVNLDAEF (sw), SEVKLDAEF, SEVKFDAEF, SEVNFDAEF, SEVKMAAEF, SEVNLAAEF, SEVKLAAEF; SEVKMLAEF, SEVNLLAEF, SEVKLLAEF, SEVKFAAEF, SEVNFAAEF, SEVKFLAEF, and SEVNFLAEF.

95. The screening kit of claim 93, wherein said substrate is selected from the group consisting of MBP-C125wt and MBP-C125sw.

96. A knock-out mouse, characterized by deletion of an endogenous ß-secretase gene.

97. The knock-out mouse of claim 96, wherein said ß-secretase gene encodes a protein having the sequence SEQ ID NO: 65.

98. The knock-out mouse of claim 97, wherein said deletion is inducible.

99. The knock-out mouse of claim 98, wherein said inducible expression is effected by a Cre-lox expression system inserted into the mouse genome.

100. A method of screening for drugs effective in the treatment of Alzheimer's disease or other cerebrovascular amyloidosis characterized by Aβ deposition, comprising

administering to a mammalian subject which is characterized by overexpression and/or deposition of Aβ a test compound selected for its ability to inhibit β-secretase activity a ß-secretase protein according to any of claims 1-10 or 22-35, and

selecting the compound as a potential therapeutic drug compound, if it reduces the amount of Aß deposition in said subject or if it maintains or improves cognitive ability in said subject.

101. The method of claim 100, wherein said mammalian subject is a transgenic mouse.

102. The method of claim 101, wherein the transgenic mouse is a PDAPP mouse or a Swedish mutation mouse.

103. A method of treating a patient afflicted with or having a predilection for Alzheimer's disease or other cerebrovascular amyloidosis, comprising

blocking the enzymatic hydrolysis of APP to Aβ in the patient by administering to the patient a pharmaceutically effective dose of a compound effective to inhibit a β-secretase protein according to any of claims 1-10 or 22-35.

104. A method of inhibiting enzymatic proteolysis of APP to Aβ in a tissue, comprising contacting said tissue with a compound effective to inhibit the enzymatic activity of a β-secretase protein according to any of claims 1-10 or 22-35.

105. The method of claim 104, wherein said inhibition of enzymatic activity is evidenced by a K_I of less than about 50 μM in a MBP-APPsw assay.

106. A therapeutic drug for the treatment of Alzheimer's disease or other cerebrovascular amyloidosis characterized by deposition of Aβ peptide, wherein said drug is selected for its ability to inhibit the enzymatic activity of a β-secretase protein according to any of claims 1-10 or 22-35.

107. The therapeutic drug of claim 106, wherein said inhibition of enzymatic activity is evidenced by a K_I of less than about 50 μM in a MBP-APPsw assay.

108. A method of diagnosing the presence of or a predilection for Alzheimer's disease in a patient, comprising

measuring β-secretase enzymatic activity in a cell sample from said patient, and

diagnosing the patient as having or having a predilection for Alzheimer's disease, if said level enzymatic activity level is significantly greater than a pre-determined control activity level.

109. The method of claim 108, wherein said measuring is carried out in a whole cell assay.

110. The method of claim 109, wherein said measuring is carried out on a β-secretase protein purified from said cell sample of said patient.

111. A method of purifying a β-secretase enzyme molecule, comprising

contacting an impure sample containing β-secretase enzyme activity with an affinity matrix which includes a β-secretase inhibitor.

112. The method of claim 111, wherein said β-secretase inhibitor is selected according to the method of any of claims 70-85.

113. The method of claim 111, wherein said β-secretase inhibitor is according to claim 89 or claim 90.